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EXAMINER	
FRONDA, C	
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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
09/341,600	Berr et al.
Examiner	**Art Unit**
Christian L. Fronda	1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☒ This action is **FINAL.** 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-39 is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-39 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some* c)☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☐ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
18) ☐ Interview Summary (PTO-413) Paper No(s). _____
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other:

DETAILED ACTION

1. Claims 1-39 are under consideration in this Office Action.

Claim Rejections - 35 U.S.C. § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

3. Claims 1-6, 8, 14-16, 18, 21 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.* in view of O'Shea *et al.*

The claims recite a fermentation method for producing a product selected from the group consisting of glucosamine and glucosamine-6-phosphate. This method is not limited toward a large industrial scale production of said product. The word "fermentation", interpreted in its broadest definition, encompasses chemical decomposition of carbohydrates in the absence of oxygen wherein said decomposition occurs in a bacterial or yeast cell.

Dutka-Malen *et al.* make a genetically engineered *E.coli* host cell (see **Fig. 1**, p. 288) which is transformed with a recombinant vector containing the *E.coli* gene encoding glucosamine-6-phosphate synthase suitably linked to a *lac* promoter wherein said host cell overexpresses glucosamine-6-phosphate synthase as evident by an increase in enzyme activity. Glucosamine-6-phosphate synthase catalyzes the formation of glucosamine-6-phosphate. Genetic modifications include transformation of *E.coli* host cells with a recombinant vector

containing a nucleic acid sequence which encodes glucosamine-6-phosphate synthase and overexpression of said synthase. Furthermore, linking the nucleic acid sequence encoding glucosamine-6-phosphate synthase to said *lac* promoter in said recombinant vector is a mutation to the glucosamine-6-phosphate synthase gene (*glms)*. Inherently, glucosamine-6-phosphate and glucosamine are produced inside said host cell and the glucosamine secreted to the culture (fermentation) medium by said host cell's glucosamine transport system.

Dutka-Malen *et al.* culture said host cell in LB medium as taught by reference to Maniatis *et al.* comprising culturing of *E. coli* in a flask which is a fementor at a temperature range from 28-37 ^{0}C wherein said flask comprises LB medium [see **Enzyme assay and purification**, p. 288; and the entire Maniatis *et al.* reference]. Dutka-Malen *et al.* harvest said host cells and disrupt the cells producing a crude extract and thereby releasing intracellular glucosamine-6-phosphate. Dutka-Malen *et al.* process this crude extract using the cited assay method taught by Zalkin (see reference number 11 in Dutka-Malen *et al.*, p.290) which comprises removing the protein precipitate in the crude extract by centrifugation (see p.279 of Zalkin). Hence, the supernate contains intracellular glucosamine-6-phosphate produced by said host cell which is purified and isolated from the crude extract.

O'Shea *et al.* teach a chromatography method for isolating glucosamine and glucosamine-6-phosphate. One of ordinary skill in the art would have used the chromatography method taught by O'Shea *et al.* and optimized said chromatography method in order to obtain glucosamine-6-phosphate or glucosamine which is to be used for commercial purposes such as dietary supplements used to treat osteoarthritis. Hence, a motivation exists to obtain glucosamine or glucosamine-6-phosphate and because of this motivation one of ordinary skill in the art would have used phosphatases to dephosphorylate the produced glucosamine-6-phosphate. In order to increase the amount of glucosamine-6-phosphate produced by said host cells taught by Dutka-Malen *et al.* to a concentration of 1g/liter product, one of ordinary skill in the art would have cultured the said host cells in terrific broth (TB) as routine optimization which increases the cell density and thereby increases the intracellular levels of glucosamine-6-phosphate (claim 9).

4. Claims 7 and 17 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Plumbridge in view of Joyce *et al.* and O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Plumbridge, Joyce *et al.*, and O'Shea *et al.*

Joyce *et al.* teach a method to delete specific genes from the *Escherichia coli* genome and the successful use of this method to delete the *polA* gene from the *Escherichia coli* genome (see entire publication). Joyce *et al.* state that the method can be applied to any gene, regardless of function and that this *in vitro* method for manipulating the target gene allows one to design a deletion that does not leave behind a functional fragment of the gene (see **Discussion**, p. 641).

Because the enzymes encoded by the genes recited in claim 39 are involved in amino sugar metabolism which deplete the amount of glucosamine-6-phosphate, one of ordinary skill in the art would have been motivated to use the method taught by Joyce *et al.* to further delete said enzymes involved in amino sugar metabolism in the *E. coli* cell taught by Dutka-Malen *et al.* in order to prevent the depletion of glucosamine-6-phosphate. The motivation to use the method taught by O'Shea *et al.* has been stated above. One of ordinary skill in the art would have been motived to obtain glucosamine or glucosamine-6-phosphate because glucosamine is to be used for commercial purposes such as dietary supplements used to treat osteoarthritis. One of ordinary skill would have had reasonable expectation of success because Joyce *et al.* successfully deleted the *polA* gene from the genome of *Escherichia coli*. and Joyce *et al.* teach that their deletion method can be applied to any gene, regardless of function and that their method for manipulating the target gene allows one to design a deletion that does not leave behind a functional fragment of the gene.

5. Claim 9 is again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of Balbas *et al.* and O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.*, Balbas *et al.*, and O'Shea *et al.*

Balbas *et al.* teach a vector for chromosomal integration of cloned DNA into the *E. coli* genome and the successful integrations of the *Vitreoscilla* sp. hemoglobin-encoding gene and the *Photobacterium leiognathi lux* genes into the *E. coli* genome (see entire publication). In addition, Balbas *et al.* teach the advantages of integration of cloned DNA into the genome of a host organism: stability of the cloned gene, and absence of undesirable copy number effects (see fist paragraph of **Introduction**, p. 65).

While the teachings of Balbas et al. are not concerned with the production of glucosamine or glucosamine-6-phosphate, Balbas et al. teach a vector, methods for using this vector to integrate genes into the *E.coli* genome, and advantages of integration of any gene into genome of a host organism which is applicable to any gene because of the successful integrations of *Vitreoscilla* sp. hemoglobin-encoding gene and the *Photobacterium leiognathi lux* genes into the *E. coli* genome. Hence, the claims are obvious over the combination of the teachings of the references of Dutka-Malen *et al.*, Balbas *et al.*, and O'Shea *et al.*

6. Claims 10 and 19 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.* and of O'Shea *et al.*

One of ordinary skill in the art would be motivated to produce glucosamine-6-phosphate

or glucosamine according to claims 10 and 19 because of the need for new cost-effective methods for production of glucosamine which is a well known, important nutritional product, i.e. as dietary supplements used to treat osteoarthritis. Furthermore, one of ordinary skill in the art would have had a reasonable expectation for success because the reagents and process steps for producing glucosamine according to claim 10 is known in the art and because of the success of Dutka-Malen *et al.* in producing a genetically modified microorganism which overexpresses glucosamine synthetase.

Glucosamine or glucosamine-6-phosphate is expected to be produced according to claims 10 and 19 by modifying the teachings of Dutka-Malen *et al.* in which the DNA encoding glucosamine synthetase is mutated by site-directed mutagenesis, UV irradiation, or treatment with mutagenic agent using methods well known in the art; the mutated DNAs are inserted into an expression vector; *E. coli* host cells are transformed by methods well known in the art with said expression vector containing the mutated DNAs; host cells are selected which contain a glucosamine synthetase that has a reduction in product inhibition by assaying for enzyme activity in the presence of product; the selected host cell is used in production of glucosamine or glucosamine-6-phosphate by contacting the selected host cell with substrates and glucosamine or glucosamine-6-phosphate isolated from the culture (fermentation) medium or from the microorganism by using the method taught by O'Shea *et al.*

7. Claims 11-13 and 20 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of Plumbridge, Joyce *et al.*, and O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.*, Plumbridge, Joyce *et al.*, and O'Shea *et al.*

A microorganism according to claims 11-13 and 20 is expected to be produced by further modifying the *E. Coli* host cell described above in the 103 rejection of claims 22, 23, 27-29, and 33 in which any gene or genes taught by Plumbridge is deleted in said host cell by using the method taught by Joyce *et al.* Deletion of any one or combination of the genes taught by Plumbridge is expected to result in a decrease or loss of enzyme activity. This host cell containing a deletion of said gene or genes is then used in production of glucosamine or glucosamine-6-phosphate by contacting the selected host cell with substrates and glucosamine or glucosamine-6-phosphate isolated from the culture (fermentation) medium or from the microorganism by using the method taught by O'Shea *et al.* One of ordinary skill in the art would be motivated to produce a microorganism according to claims 11-13 and 20 for use in the method of claim 1 or 18 because of the need for new cost-effective methods for production of glucosamine which is a well known, important nutritional product used as dietary supplements used to treat osteoarthritis.

8. Claims 22, 23, 27-29, and 33 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of Balbas *et al.* and O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.*, Plumbridge, Joyce *et al.*, and O'Shea *et al.*

A microorganism according to claims 22, 23, 27-29, and 33 is expected to be produced by modifying the teachings of Dutka-Malen *et al.* in which the DNA encoding glucosamine synthetase is mutated by site-directed mutagenesis, UV irradiation, or treatment with mutagenic agent using methods well known in the art; the mutated DNAs are inserted into the integrative vector taught by Balbas *et al.* and the recombinant, integrative vector is used to transform an *E. coli* host cell.; and host cells are selected which contain a glucosamine synthetase that has a reduction in product inhibition by assaying for enzyme activity in the presence of product. Because methods are well known to increase the cell density of *E. coli* in culture such as culturing in TB (terrific broth), glucosamine is expected to be produced at a concentration of at least 1 g/L when expression of glucosamine-6-phosphated synthase is induced by 1 mM IPTG and the products recovered by using the method taught by O'Shea *et al.* (*cf* claim 33).

One of ordinary skill in the art would be motivated to produce glucosamine according to claims 22, 23, 27-29, and 33 because of the need for new cost-effective methods for production of glucosamine which is a well known, important nutritional product used as dietary supplements used to treat osteoarthritis.

9. Claims 24-26, and 30-32 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of Balbas *et al.*, Plumbridge , and Joyce *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.*, Balbas *et al.*, Plumbridge , and Joyce *et al.*

A microorganism according to claims 24-26, and 30-32 is expected to be produced by further modifying the *E. Coli* host cell described above in the 103 rejection of claims 22, 23, 27-29, and 33 in which any gene taught by Plumbridge is deleted in said host cell by using the method taught by Joyce *et al.* Deletion of any one or combination of the genes taught by Plumbridge is expected to result in a decrease or loss of enzyme activity. One of ordinary skill in the art would be motivated to produce a microorganism according to claims 24-26, and 30-32 because of the need for new cost-effective methods for production of glucosamine which is a well known, important nutritional product used as dietary supplements used to treat osteoarthritis.

10. Claims 34-39 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Dutka-Malen *et al.* in view of Balbas *et al.*, Plumbridge, Joyce *et al.*, and O'Shea *et al.* Applicants' arguments filed on October 19, 2000, have been fully considered but they are not persuasive. Applicants argue that the claims are not obvious over the combined teachings of the references of Dutka-Malen *et al.*,Balbas *et al.*, Plumbridge, Joyce *et al.*, and O'Shea *et al.*

A microorganism is expected to be produced according to claims 34-39 in which the *E. coli* glucosamine synthetase gene taught by Dutka-Malen *et al.* is inserted into the integrative vector taught by Balbas *et al.*; the recombinant, integrative vector is used to transform *E. coli* host cells (*cf.* claim 34); host cells are selected which have increased expression of enzyme activity; and any gene taught by Plumbridge is deleted in said host cell having increased expression of enzyme activity by using the method taught by Joyce *et al.* Deletion of any of the genes taught by Plumbridge is expected to result in a decrease or loss of enzyme activity. Because methods are well known to increase the cell density of *E. coli* in culture such as culturing in TB (terrific broth), glucosamine is expected to be produced at a concentration of at least 1 g/L when expression of glucosamine-6-phosphated synthase is induced by 1 mM IPTG and the products recovered by using the method taught by O'Shea *et al.* (*cf* claim 39).

One of ordinary skill in the art would be motivated to produce a microorganism according to claims 34-39 because of the need for new cost-effective methods for production of glucosamine which is a well known, important nutritional product used as dietary supplements used to treat osteoarthritis. Furthermore, one of ordinary skill in the art would have had a reasonable expectation for success because Dutka-Malen *et al.* succeeded in producing a genetically modified microorganism which overexpresses glucosamine-6-phosphate synthase and Joyce *et al.* successfully deleted the *polA* gene from the genome of *Escherichia coli.*

Conclusion

11. No claim is allowed.

12. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event,

however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christian L. Fronda whose telephone number is (703)305-1252. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703)308-3804. The fax phone number for this Group is (703)308-0294. Any inquiry of a general nature or relating to the status of this application should be directed to the Group 1600 receptionist whose telephone number is (703)308-0196.

CLF

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